Fragmentation of Singly-charged Peptide Derivatives Produced by Atmospheric Pressure Matrix-assisted Laser Desorption Ionization

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Atmospheric Pressure MALDI (AP-MALDI) forms mainly singly-charged ions, providing a means for simplifying peptide mixture analysis using MS^n capabilities of a quadrupole ion trap[1-2]. However, many singly-charged peptides do not readily fragment at the collision energies employed in a quadrupole ion trap (typical ion trap experiments target ES multiply-charged peptides). A recently developed derivitization method[3] creates a negative charge at the N-terminus, resulting in the delocalization of the proton (added during the ionization process) along the peptide backbone. The ion is more readily fragmented, producing mostly y-type ions. The approach targets Arg or Lys containing peptides of tryptic digests, enhancing singly-charged AP-MALDI peptide fragmentation in quadrupole ion traps.

METHODS

Several peptides and a cytochrome c tryptic digest were derivatized using a procedure published earlier[3]. Briefly, a sulfonic acid group is attached to the N-terminus of the peptides with the reagent chlorosulfonylacetyl chloride. Parent and derivative compounds were introduced into the ion trap using home built electrospray and AP MALDI ion sources interfaced to a Finnigan ion trap mass spectrometer. Standard isolation and excitation procedures were used to produce MS/MS spectra. Ions produced by both methods were fragmented to compare the threshold energies for fragmentation and to compare the resultant fragmentation patterns.

RESULTS AND DISCUSSION

The sulfonic acid derivitization procedure was used to generate singly-charged derivatives of peptides, as evidenced by AP-MALDI and ES mass analysis. Ions were characterized by addition of one or more sulfonic acid groups, denoted by mass shifts of +122 Da from the parent peptide. Derivative ions were then isolated and fragmented in the ion trap. A dramatic difference in the fragmentation behavior was observed with several peptides, including Angiotensin I and Substance P. The singly-charged ions formed by AP-MALDI and ES do not easily fragment in the ion trap, and provide only minimal structural information. However, the addition of a strong acid group to the N-terminus suppresses the N terminal fragmentation and promotes C terminal fragmentation yielding y-ions. Consequently, MS/MS analysis of ES and AP-MALDI ionized Angiotensin I derivative produced the y-ion series from y4 to y9. Figure 1 demonstrates the lack of fragmentation information obtained from AP-MALDI MS/MS analysis of Substance P. The MS/MS analysis of the Substance P derivative gives valuable information about the peptide sequence; however, it does not follow the expected y-ion series fragmentation pattern. The Substance P analyzed (obtained from Sigma) contains an amide at the C-terminus causing the sulfonic acid group to attach to the C-terminus rather than the N-terminus. Although the expected y-ion series is not produced, the derivative does provide sequence information for Substance P, as seen in Figure 2. The MS/MS analysis of the peptide ASHLGLAR exhibits several fragment ion types (a, b, y), producing a complicated spectrum (not shown). However, fragmentation of the ASHLGLAR derivative leads to a simpler spectrum of the y-ion series (y3 to y7). Simplification of the fragmentation pattern is beneficial in determining the peptide sequence of unknown analytes. As expected, the effect of adding the sulfonic acid group does not lower the activation energy for fragmentation enough to allow for large peptides, such as the Insulin Chain B derivative, to be fragmented.

The most significant potential use for this approach may be in the analysis of tryptic digests. The tryptic digest of cytochrome c produces several peptides that do not fragment well as singly-charged AP-MALDI ions. For example, AP-MALDI MS/MS spectrum of fragment TGPNLHGLFGR is seen in Figure 3. For comparison, Figure 4 demonstrates that fragmentation of the derivative yields the y-ion series of y5, y6, y9, and y10. Not all the peptides derivatized and analyzed produced better fragmentation spectra. Future studies will focus on the application of this method for other peptides.

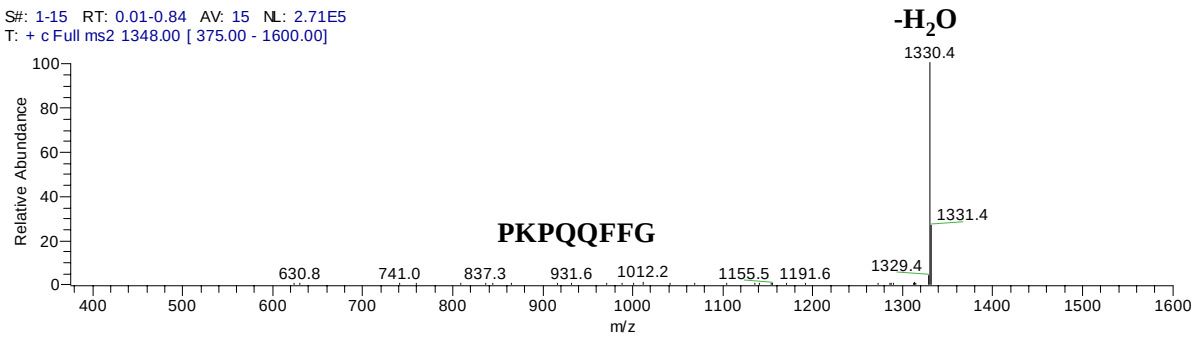

FIGURE 1. The AP-MALDI MS/MS spectrum of Substance P gives little peptide sequence information.

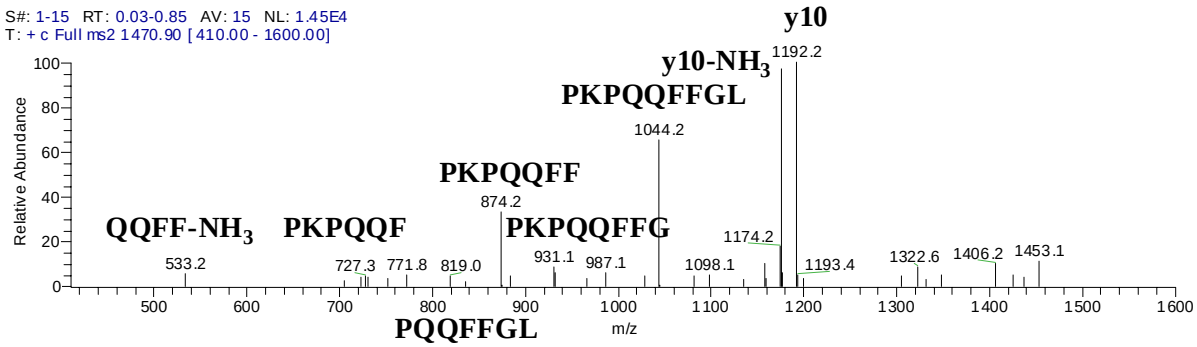

FIGURE 2. The AP-MALDI MS/MS spectrum of the Substance P derivative provides useful information.

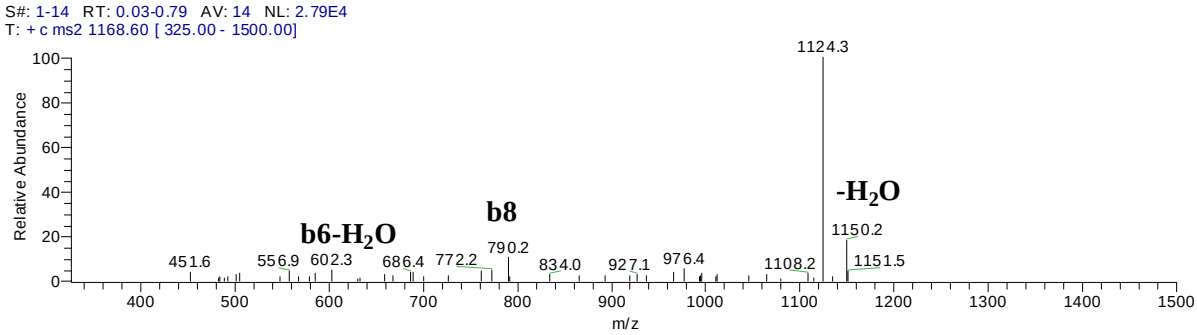

FIGURE 3. AP-MALDI MS/MS of Cytochrome C tryptic fragment TGPNLHGLFGR does not yield y-ions.

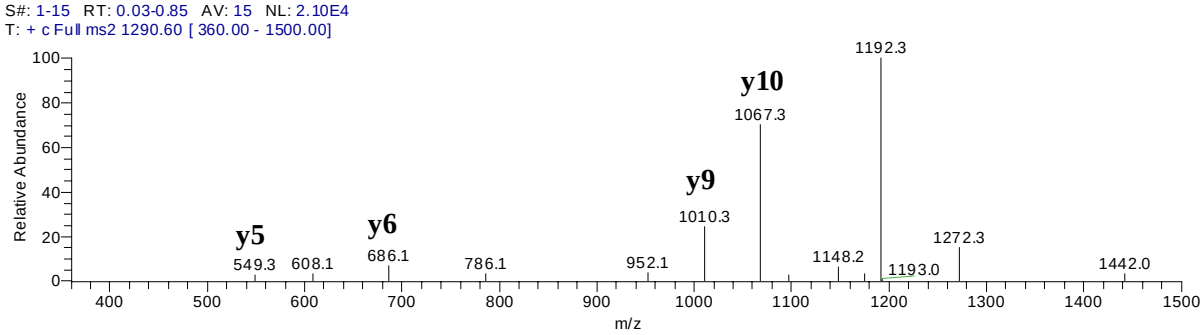

FIGURE 4. By derivatizing TGPNLHGLFGR, y-ions can be produced and detected by AP-MALDI MS/MS.

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[2] V.V. Laiko, S.C. Moyer, and R.J. Cotter, *Anal. Chem.*, 2000, **72**, 5239-5243.
[3] T. Keough, R.S. Youngquist, and M.P. Lacey, *Proc. Natl. Acad. Sci. USA*, 1999, **96,** 7131-7136.